PREPARATION OF SRM 967 CREATININE IN HUMAN SERUM

Prepared at Solomon Park Research Institute, Kirkland, Washington

Human serum with the following characteristics:

Master Pool

Comprised of units drawn from postmenopausal female donors to yield pool with creatinine of 0.8 mg/dL or lower.

Collected and handled following NCCLS C37-A

All units tested and found negative for viral markers as specified by FDA

Pooled and split into two sub-pools

PREPARATION OF SRM 967 *continued*

For Low level, 1200 1 mL aliquots prepared in 2 mL amber vials

For High level, second sub-pool spiked with crystalline creatinine to bring the concentration to 4.0 mg/dL ± 0.2mg/dL.

1200 1 mL aliquots prepared in 2 mL amber vials

LC/MS METHOD FOR SERUM CREATININE

Based on published method: [P. Stokes and G. O'Connor, J., Chromatog. B 794, 125-136 (2003)]

Method in brief:

Spike serum with creatinine-d_3
Precipitate proteins with cold ethanol
Centrifuge
Decant supernatant and dry under nitrogen
Reconstitute in water and filter
Dilute with 10 mM ammonium acetate

LC/MS
Phenomenex LUNA C-18
Gradient: 10 mM ammonium acetate for 7 min,
Then acetonitrile:10 mM ammonium acetate (80:20) and hold for 13 min

Electrospray ionization – positive mode monitoring $(M+H)^+$ at 114/117

Calibration from standard curve using SRM 914a and creatinine- d_3

MEASUREMENT OF SRM 967 USING LC/MS

3 Sets, 3 Vials of Each Level per Set, 2 Aliquots/vial

(mg/dL)

Set	Box	Vial	Sample	Concentration	Set Mean	Set St. Dev.	Set CV, %
SRM 967 Level I							
1	3	39	1	0.749	0.747	0.001	0.180
1	3	39	2	0.748			
1	13	7	3	0.746			
1	13	7	4	0.748			
1	18	49	5	0.746			
1	18	49	6	0.746			
2	4	12	15	0.747	0.747	0.002	0.251
2	4	12	16	0.750			
2	14	22	17a	0.749			
2	14	22	18a	0.745			
2	15	39	19	0.747			
2	15	39	20	0.745			
3	6	13	29	0.749	0.748	0.002	0.315
3	6	13	30	0.750			
3	11	44	31	0.746			
3	11	44	32	0.750			
3	22	28	33	0.746			
3	22	28	34	0.745			
Mean				**0.747**			
St. Dev.				**0.002**			
CV, %				**0.243**			

SUMMARY RESULTS FOR LC/MS

Level	Mean	CV%	Certified Value
967 level I	0.747	0.24	
967 level II	3.918	0.24	
909b level I	0.632	0.67	0.6355 ± 0.0062
909b level II	5.275	0.23	5.287 ± 0.060

GC/MS MEASUREMENTS OF SRM 967

NIST ID-GC/MS “Definitive Method”

Measurements are starting in August

New staff member is responsible for setting up method

PROPOSED COMMUTABILITY STUDY

Materials

20 fresh frozen patient specimens

SRM 967 – two levels

CAP LN 24 materials

SRM 909b – two levels

Methods

NIST LC/MS method

U. Minnesota (J. Eckfeldt) field methods
(CX3, Roche Jaffe, Roche Enzymatic, Vitros

Other Participants?